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Vol. 10: 363–368, 2018
https://doi.org/10.3354/aei00273

AQUACULTURE ENVIRONMENT INTERACTIONS
Aquacult Environ Interact

Published August 1

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NOTE

Acute toxic effects of hydrogen peroxide, used for salmon lice treatment, on the survival of polychaetes *Capitella* sp. and *Ophryotrocha* spp.

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ABSTRACT: The amount of hydrogen peroxide (H_2O_2) used in the treatment of salmon lice in Norwegian salmon farming increased from 308 tons in 2009 to 43 246 tons in 2015. For 2016 and 2017, however, the consumption was reduced to 26 597 and 9277 tons, respectively. The use of this compound may have negative impacts on benthic fauna underneath the fish farms and, in particular, on polychaetes, which can be found in large numbers at the bottom under fish farms where they play a key role in the turnover of organic waste from the farm. The tolerance of *Capitella* sp. and *Ophryotrocha* spp. to a 1 h exposure to H_2O_2 (0, 100, 200, 400, 800, 1200 and 1800 mg l^{-1}) was evaluated. The recommended dose for treatment of the salmon is 1800 mg l^{-1}. Following exposures, the polychaetes were reintroduced into clean sea water. Both polychaete species experienced high cumulative mortality during a 72 h post-exposure period. The mortality showed to be dose dependent, with the highest dose giving the highest mortality. The 50% lethal concentration (LC_{50}) of *Capitella* sp. was significantly higher than the LC_{50} of *Ophryotrocha* spp. at the same exposure time ($p < 0.05$). The 50% lethal time of *Capitella* sp. was significantly longer than that of *Ophryotrocha* spp. at the same concentration ($p < 0.05$). The results show that 1 h exposures to H_2O_2 at all the tested concentrations had irreversible negative effects on both polychaete species.

KEY WORDS: Hydrogen peroxide · Polychaete · Acute toxicity · Mortality · LC_{50}

INTRODUCTION

The ectoparasite salmon louse *Lepeophtheirus salmonis* (Krøyer 1837) is a major problem for the cage farming of Atlantic salmon *Salmo salar* in Norway. A number of compounds are therefore available to combat the parasite, including chitin synthesis inhibitors, acetylcholinesterase inhibitors, pyrethroids, avermectins and the antiseptic hydrogen peroxide (H_2O_2). Because of the reduced sensitivity of salmon lice to pyrethroids and emamectin and the emergence of amoebic gill disease in Norwegian salmon farming, the use of H_2O_2 has steadily increased since 2009, reaching a total of 43 246 tons in 2015 (Adams et al. 2012, Grøntvedt et al. 2015). In the last 2 yr, however, the consumption has been reduced to 26 597 tons in 2016 and further to 9277 tons in 2017 (www.fhi.no/). H_2O_2 is used as bath treatment, and prior to administration, the water volume in the cage is temporarily reduced and the cage surrounded by a tarpaulin. The

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H_2O_2 is added to the cage to a final bath concentration of 1500 to 1800 mg l^{-1} depending on temperature, and the exposure time is 20 to 30 min. Following treatment, the tarpaulin is removed and the released H_2O_2 may disperse both vertically and horizontally. Since a bath solution of H_2O_2 is slightly heavier than the surrounding sea water, modeling has shown that the plume may sink after release if the water column is homogeneous (Refseth et al. 2016). This was confirmed by Fagereng (2016), who, in a field investigation, found up to 724 mg l^{-1} of H_2O_2 at a depth of 60 m and detectable concentrations even at 130 m. Coupled with a predicted no-effect concentration of 0.01 mg l^{-1} determined for H_2O_2 in water (Institute for Health and Consumer Protection in Finland 2003), the results of Fagereng (2016) suggest that the use of H_2O_2 in salmon farming may be harmful to non-target organisms located near salmon farms and, given the right conditions, benthic organisms like polychaetes may also be exposed.

H_2O_2 has long been regarded as an environmentally friendly salmon lice agent because it readily dissociates to water and oxygen. However, Fagereng (2016) calculated degradation half-lives of 28 and 3.5 d of H_2O_2 in seawater at temperatures of 8.7 and 12°C, respectively. Furthermore, it has been shown that low concentrations (<0.170 mg l^{-1}) of H_2O_2 affect the physiology of aquatic animals, such as antioxidant enzyme activities in the polychaetes *Arenicola marina* and *Nereis* (*Hediste*) *diversicolor* (Abele-Oeschger et al. 1994, Buchner et al. 1996). Effects on oxygen consumption, catalase and glutathione peroxidase activity were further seen in the polychaete *Laeonereis acuta* when exposed to concentrations of 0.34 and 1.7 mg l^{-1} for up to 10 d (da Rosa et al. 2008). The acute effects of high concentrations of H_2O_2 on aquatic invertebrates are largely unknown, but Fagereng (2016) showed that 1 h exposure of pink shrimps *Pandalus montagui* to 170 mg l^{-1} led to reduced flight response even after a 24 h recovery period. A 1 h exposure of copepods *Calanus* sp. gave a 50% lethal concentration (LC_{50}) of less than 5% of the recommended dose of 1700 mg l^{-1} (Escobar-Lux 2016). On the other hand, a 1 h exposure gave an LC_{50} higher than 1700 mg l^{-1} for sand shrimp *Crangon septemspinosa*, rock pool shrimp *Palaemon elegans*, chameleon shrimp *Praunus flexuosus* and adult American lobster *Homarus americanus* (Burridge et al. 2014, Brokke 2015). Hence, major differences in sensitivity between species are seen. Therefore, there is a need for more knowledge about the effect of H_2O_2 exposure on non-target organisms, especially for benthic species.

Polychaetes are naturally abundant in benthic habitats under fish farms and in other types of anthropogenically modified estuaries (Kutti et al. 2007, Dafforn et al. 2013, Bannister et al. 2014). Opportunistic polychaetes that are adapted to nutrient-rich habitats and commonly found underneath fish farms located over hard bottom in Norway include *Vigtorniella ardabilia* and *Ophryotrocha* spp. (Paxton & Davey 2010, R. Bannister pers. comm.) and over soft-sediment areas, *Capitella* sp. (Kutti et al. 2007, Dean 2008). The polychaetes are important in environmental recovery by consuming and transforming the organic materials deposited from the fish farm (Dean 2008). Because these species live near the fish farms, they may be exposed to agents originating from activities at the farm, including salmon lice treatment. The objective of this study was therefore to find the limit of tolerance of *Capitella* sp. and *Ophryotrocha* spp. to short time exposure to H_2O_2. This will contribute to the evaluation of the effects of sea lice drugs on the natural environment surrounding fish cages.

MATERIALS AND METHODS

Animal collection and acclimatization

Capitella sp. were collected by grab sampling (250 cm^2) underneath a fish farm located at Austevoll, Norway. *Ophryotrocha* spp. were collected at a fish farm in Hardangerfjord, Norway, using artificial plastic grass mounted in an iron frame of 1.2 × 1.2 × 0.1 m and deployed underneath a fish cage for 2 wk. The collected polychaetes under the fish farms were representative species. At both fish farms, alternative methods (fresh water, increased water temperature) had recently been used for delousing purposes. Directly after sampling, polychaetes were placed in boxes containing sea water collected from about 150 m depth. The boxes, supplied with air, were transported to the laboratory at Austevoll Research Station (Institute of Marine Research, Norway). *Capitella* sp. specimens were placed in four 100 l tanks, with 1 kg of glass beads (6 mm diameter) in each tank mimicking artificial benthic substrate. The *Ophryotrocha* spp. were placed in 100 l tanks, each supplied with 5 stones of about 300 g serving as substrate. The stones facilitated aggregation of *Ophryotrocha* spp. and provided a rough substrate to attach mucus strings, mimicking a hard-bottom substrate. Tanks were supplied with a seawater flow of 1150 to 1500 ml min^{-1} from 150 m depth holding

a temperature of 8 to 9°C. The polychaetes were acclimatized for 5 d and fed ground salmon pellets produced by Skretting once a day. The tanks were kept in darkness during the acclimation period, except during feeding.

Experimental design

Polychaetes were exposed to 6 nominal concentrations of H_2O_2 (100, 200, 400, 800, 1200, 1800 mg l^{-1}) for 1 h, where the highest concentration is equal to the recommended dose used for treatment. Concentrations were prepared by diluting the stock formulation (Nemona 49, 5%, Akzo Nobel) with sea water to the desired concentration for each treatment. The polychaetes (>50 individuals, estimated from pre-calculated volume per numbers) were transferred to 2 l beakers containing the decided concentration of H_2O_2. Beakers without H_2O_2 served as controls. Three replicate groups were used for each concentration, including control groups. Following exposure (1 h), the H_2O_2 solution in the beaker was replaced with clean water and a continuous flow (150–180 ml min^{-1}) of sea water established. The number of dead animals was recorded at 1, 6, 12, 24, 48 and 72 h from the start of H_2O_2 exposure; the number of remaining survivors was also counted at 72 h. Beakers were kept in the dark during the experimental period.

Statistical analysis

The 50% lethal time (LT_{50}) is the time where 50% of the organisms have died after exposure to a toxic substance or stressful condition. The LC_{50} refers to the concentration for half the population to die from a treatment or exposure. The LC_{50} and LT_{50} values were calculated by the Bliss Probit Method (Sprague 1969). Data were analyzed using the SPSS for Windows (Version 13.0) statistical package. The calculated LC_{50} and LT_{50} values are presented as mean ± SD, unless stated otherwise. The differences in mortalities for each treatment and time intervals were assessed with 1-way ANOVA followed by Duncan's multiple range tests for post hoc pairwise comparisons. A dependent *t*-test was applied to detect any differences between LT_{50} and LC_{50} for the 2 species. Curve estimation was used to analyze the relationship between LC_{50} and time and between LT_{50} and concentrations. Differences were statistically significant if $p < 0.05$.

RESULTS

Mortality

For *Capitella* sp., no mortality was seen in the control groups. In the H_2O_2-exposed groups, the acute mortality after 1 h exposure was dose dependent, with the 2 highest doses giving a mortality of >60%. A delay in mortality, on the other hand, was seen for the 100, 200 and 400 mg l^{-1} doses (Fig. 1a). The cumulative mortality increased gradually throughout the experimental period of 72 h, reaching over 90% for all doses except for 100 mg l^{-1}, which reached 76% (Fig. 1a).

The sensitivity of *Ophryotrocha* spp. to H_2O_2 was significantly higher than that of *Capitella* sp. A 1 h exposure resulted in acute mortality for all doses, reaching 100% for the 1200 and 1800 mg l^{-1} doses and 20% for the 100 mg l^{-1} dose (Fig. 1b). After 72 h, the cumulative mortalities were nearly 100% for all doses (Fig. 1b). Some mortality was registered in the control beaker but was less than 10% after 72 h.

LC_{50} and LT_{50}

A significant relation was found between LC_{50} and exposure time for both *Capitella* sp. and *Ophryotrocha* spp. ($p < 0.05$, Fig. 2). The curve estimation analysis for the LC_{50} of *Capitella* sp. showed a higher

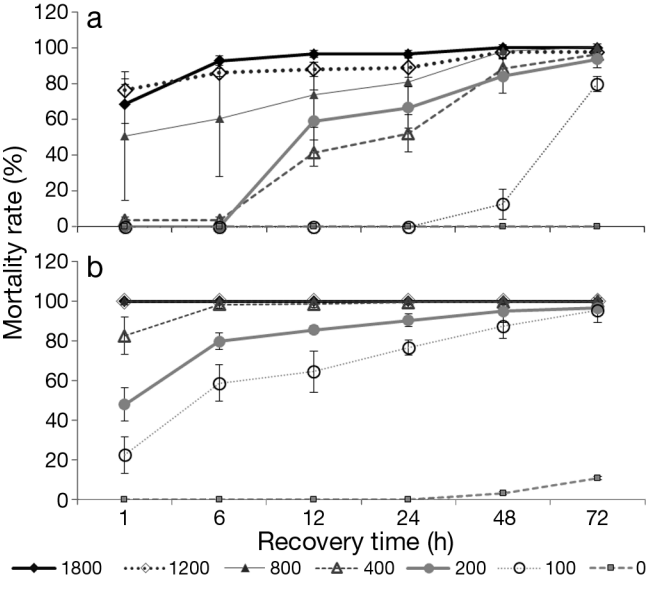

Fig. 1. Average mortality of (a) *Capitella* sp. and (b) *Ophryotrocha* spp. at exposure concentrations (0, 100, 200, 400, 800, 1200, 1800 mg l^{-1}, n = 3) of hydrogen peroxide. Error bars represent 1 SD

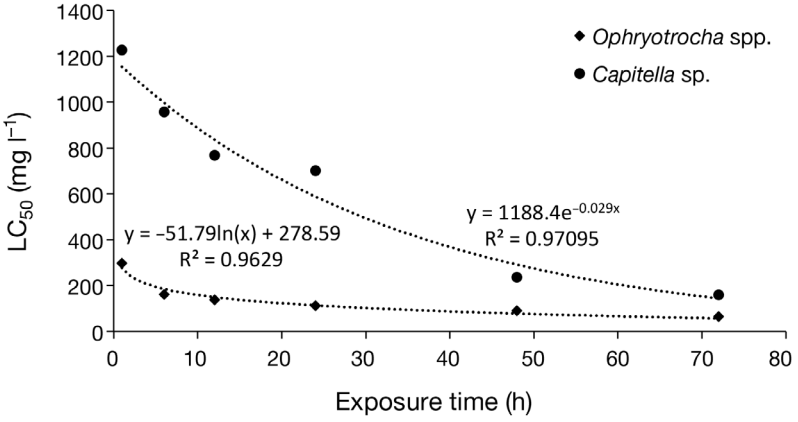

Fig. 2. Lethal concentration (LC_{50}, mg l^{-1}) of the polychaete species *Capitella* sp. and *Ophryotrocha* spp. exposed to hydrogen peroxide at increasing time intervals after exposure. The equations stem from the results of curve estimation analysis of the relationship between LC_{50} and time

R^2 value in an exponential model than in the logarithmic one. The LC_{50} of *Capitella* sp. was significantly higher compared to *Ophryotrocha* spp. at each time interval post exposure (*t*-test, $p < 0.05$). The LC_{50} of *Capitella* sp. at 1 h was 1227 mg l^{-1}, and the corresponding value for *Ophryotrocha* spp. was 296 mg l^{-1}, i.e. 4 times lower. At 72 h post exposure, the difference was reduced, with a calculated LC_{50} of 159.3 mg l^{-1} for *Capitella* sp. and 64.3 mg l^{-1} for *Ophryotrocha* spp.

Similarly, a significant relation was observed between LT_{50} and H_2O_2 concentrations for both species ($p < 0.05$, Fig. 3). The LT_{50} for *Capitella* sp. with 76, 32, 33 and 11 h for the doses 100, 200, 400 and 800 mg l^{-1}, respectively, was significantly longer compared to *Ophryotrocha* spp., with an LT_{50} of 24 and 4 h for doses of 100 and 200 mg l^{-1}, respectively (*t*-test, $p < 0.05$). The results thus indicate that more

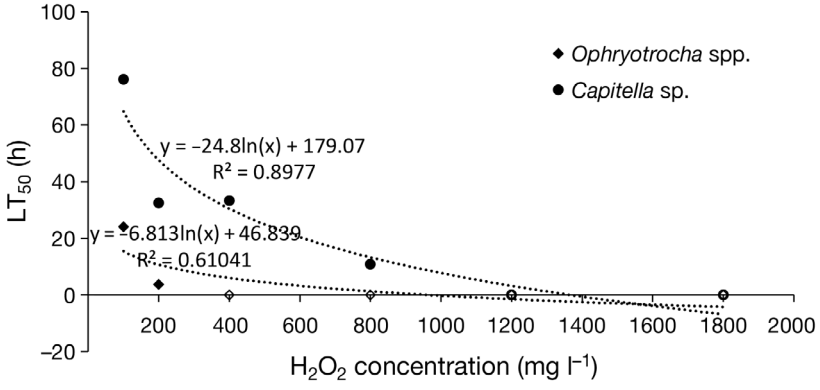

Fig. 3. Lethal time (LT_{50}, h) of 2 polychaete species (*Capitella* sp. and *Ophryotrocha* spp.) exposed to different hydrogen peroxide (H_2O_2) concentrations. The markers without filling mean the LT_{50} is less than 1 h. The equations stem from the results of curve estimation analysis of the relationship between LT_{50} and concentration

than 50% of the *Ophryotrocha* spp. population would not survive 1 h exposure to H_2O_2 if the concentration exceeded 400 mg l^{-1}. For *Capitella* sp., a concentration of 1200 mg l^{-1} would result in 50% mortality within 1 h.

DISCUSSION

Even with an exposure time of only 1 h, both *Capitella* sp. and *Ophryotrocha* spp. showed low acute tolerance to the recommended dose of H_2O_2 used for delousing. Both species also uncovered limited capacity to recover after exposure to all concentrations tested. An observed effect in both species was change in skin color. The skin of *Capitella* sp. turned from red to gray during the exposure, and the skin of *Ophryotrocha* spp. turned from light red to white. This was particularly clear for the high concentrations. Most of the polychaetes that were alive after exposure did not survive the recovery period. Therefore, it seems that the damage from H_2O_2 exposure is irreversible in both species and leads to high mortality even at doses that are realistic and ecologically relevant. Further studies should therefore include even lower doses of H_2O_2 and be combined with a longer recovery period and studies of sublethal effect parameters. The mortality after 1 h exposure was considerably lower in *Capitella* sp. than in *Ophryotrocha* spp. However, this difference was reduced at the end of the experiment, as both species experienced a substantial mortality in the recovery period. This highlights the importance of including an extended recovery period when studying compounds like H_2O_2. Low H_2O_2 concentrations (<2.0 mg l^{-1}) have previously been shown to cause adverse effects on polychaetes (Abele-Oeschger et al. 1994, Buchner et al. 1996, da Rosa et al. 2008). As *Capitella* sp. inhabit benthic sediments and may show behavioral avoidance via burrowing, this may affect the exposure to H_2O_2 discharged from the farms. However, analytical challenges make it difficult to definitively determine whether discharged H_2O_2 might infiltrate the sediment, making burrowing less advantageous. More work should therefore be done to study the effects of H_2O_2 on the *Capitella* sp. when settled in a sediment. For the *Ophryotrocha* spp. living on the surface of

the substrate, the situation can indeed be much more critical.

The calculated LC_{50} of 159.3 mg l^{-1} for *Capitella* sp. and 64.3 mg l^{-1} for *Ophryotrocha* spp. resembles that for *Calanus* sp., with an LC_{50} lower than 5% of the recommended dose of 1700 mg l^{-1} (Escobar-Lux 2016). In comparison, Burridge et al. (2014) estimated the LC_{50} of American lobster larvae (stage 1) to be 1637 mg l^{-1} after 1 h exposure and 96 h recovery time. Corresponding LC_{50} values for sand shrimp and the chameleon shrimp were 3182 and 937 mg l^{-1}, respectively (Burridge et al. 2014). Using a maximum dose of 1700 mg l^{-1} of H_2O_2 and 1 h exposure followed by 24 h recovery, no LC_{50} could be generated for chameleon shrimp and grass prawns, as the cumulative mortality never exceeded 10% (Brokke 2015). In comparison, Uc-Peraza & Delgado-Blas (2015) found that *Capitella* sp. C was less sensitive to anionic surfactants than the freshwater crustaceans *Ceriodaphnia* cf. *dubia* and *Daphnia magna* and the Nile tilapia *Oreochromis niloticus* but more sensitive than the Malaysian trumpet snail *Melanoides tuberculata*. In the studies of Burridge et al. (2014), Brokke (2015), Escobar-Lux (2016) and Fagereng (2016), either the commercial H_2O_2 formulation Nemona (Akzo Nobel) or Paramove (49.5%, Solvay Chemicals International) was used. In comparison, Hansen et al. (2017) used Perhydrol, a 30% pro-analysis product from Merck, in their study. Whether there may be differences in the toxicity, first between the 2 anti-sea lice products and second between those products and the pro-analysis product, has to our knowledge not been investigated.

Following treatment, the H_2O_2 is released and will disperse horizontally and vertically, if the water column is homogeneous, since a bath solution of H_2O_2 is slightly heavier than the surrounding sea water (Refseth et al. 2016). Fagereng (2016), in one of a very limited number of field studies, reported vertical distribution of H_2O_2, finding concentrations of 271 to 723 mg l^{-1} at a depth of 60 m for nearly 20 min at one sampling station but also horizontal distribution, where the drug was found in the upper 30 m and at concentrations up to 69 mg l^{-1}. The discharged H_2O_2 from fish farms is therefore likely to be harmful for the polychaetes underneath and in the proximity of the fish farms.

Referring to Sprague (1971), the safe concentration of H_2O_2 is assumed to represent 1% of LC_{50} at 72 h for estimation of chronic, sublethal and cumulative H_2O_2 toxicity. Using this assumption, the safe concentrations of H_2O_2 to *Capitella* sp. and *Ophryotrocha* spp. will be 1.59 and 0.64 mg l^{-1}, respectively.

H_2O_2 has long been regarded as the most environmentally friendly anti-salmon lice agent. This study demonstrates nonetheless that the potential risk of H_2O_2 to aquatic organisms is obvious and serious and therefore requires more attention in research and legislation than previously assumed. It is thereby important to define relevant concentrations of H_2O_2 in the environment. Further research should also include non-lethal stress responses and different life stages of the organisms tested.

Acknowledgements. We thank Lerøy Vest AS and Marine Harvest AS for providing access to the farms and help with polychaete collection. Thanks to Anders Mangor-Jensen and Michal Reymer for assistance during the experiment. This research was supported by the Research Council of Norway (263870/H30); Ocean Forest AS (R&D license project); Environment and Aquaculture Governance (MFA, CHN 2152); Central Public-interest Scientific Institution Basal Research Fund, Chinese Academy of Fishery Science (2018GH15); and Laboratory for Marine Fisheries Science and Food Production Processes, Qingdao National Laboratory for Marine Science and Technology, PR China (2016LMFS-A18).

LITERATURE CITED

Abele-Oeschger D, Oeschger R, Theede H (1994) Biochemical adaptations of *Nereis diversicolor* (Polychaeta) to temporarily increased hydrogen peroxide levels in intertidal sandflats. Mar Ecol Prog Ser 106:101–110

Adams MB, Crosbie PBB, Nowak BF (2012) Preliminary success using hydrogen peroxide to treat Atlantic salmon, *Salmo salar* L., affected with experimentally induced amoebic gill disease (AGD). J Fish Dis 35:839–848

Bannister RJ, Valdemarsen T, Hansen PK, Holmer M, Ervik A (2014) Changes in benthic sediment conditions under an Atlantic salmon farm at a deep, well-flushed coastal site. Aquacult Environ Interact 5:29–47

Brokke KE (2015) Mortality caused by de-licing agents on the non-target organisms chameleon shrimp (*Praunus flexuosus*) and grass prawns (*Palaemon elegans*). MSc dissertation, University of Bergen

Buchner T, Abele-Oeschger D, Theede H (1996) Aspects of antioxidant status in the polychaete *Arenicola marina*: tissue and subcellular distribution, and reaction to environmental hydrogen peroxide and elevated temperatures. Mar Ecol Prog Ser 143:141–150

Burridge LE, Lyons MC, Wong DKH, MacKeigan K, VanGeest JL (2014) The acute lethality of three anti-sea lice formulations: AlphaMax®, Salmosan®, and Interox® Paramove™ 50 to lobster and shrimp. Aquaculture 420–421:180–186

da Rosa CE, Bianchini A, Monserrat JM (2008) Antioxidant responses of *Laeonereis acuta* (Polychaeta) after exposure to hydrogen peroxide. Braz J Med Biol Res 41: 117–121

Dafforn KA, Kelaher BP, Simpson SL, Coleman MA and others (2013) Polychaete richness and abundance enhanced in anthropogenically modified estuaries despite high

concentrations of toxic contaminants. PLOS ONE 8: e77018

Dean HK (2008) The use of polychaetes (Annelida) as indicator species of marine pollution: a review. Rev Biol Trop 56:11–38

Escobar-Lux R (2016) The effect of an anti sea lice therapeutant, hydrogen peroxide, on mortality, escape response and oxygen consumption on *Calanus* spp. MSc dissertation, Pierre and Marie Curie University, Paris

Fagereng MB (2016) Use of hydrogen peroxide in aquaculture dilution studies and effects on flower prawns (*Pandalus montagui*). MSc dissertation, University of Bergen

Grøntvedt RN, Jansen PA, Horsberg TE, Helgesen K, Tarpai A (2015) The surveillance programme for resistance to chemotherapeutants in salmon lice (*Lepeophtheirus salmonis*). Surveillance programmes for terrestrial and aquatic animals in Norway. Annual report 2014. Norwegian Veterinary Institute, Oslo

Hansen BH, Hallmann A, Altin D, Jenssen BM, Ciesielski TM (2017) Acute hydrogen peroxide (H_2O_2) exposure does not cause oxidative stress in late-copepodite stage of *Calanus finmarchicus*. J Toxicol Environ Health A 80:820–829

Institute for Health and Consumer Protection in Finland (2003) Hydrogen peroxide: summary risk assessment report. European Communities, Helsinki

Kutti T, Hansen PK, Ervik A, Høisæter T, Johannessen P (2007) Effects of organic effluents from a salmon farm on a fjord system. II. Temporal and spatial patterns in infauna community composition. Aquaculture 262:355–366

Paxton H, Davey A (2010) A new species of *Ophryotrocha* (Annelida: Dorvilleidae) associated with fish farming at Macquarie Harbour, Tasmania, Australia. Zootaxa 2509: 53–61

Refseth GH, Sæther K, Drivdal M, Nøst OA and others (2016) Miljørisiko ved bruk av hydrogenperoksid. Økologisk vurdering og grenseverdi for effekt. Rapport 8200-1. Akvaplan-niva, Trondheim

Sprague JB (1969) Measurement of pollutant toxicity to fish I. Bioassay methods for acute toxicity. Water Res 3:793–821

Sprague JB (1971) Measurement of pollutant toxicity to fish—III: sublethal effects and 'safe' concentrations. Water Res 5:245–266

Uc-Peraza RG, Delgado-Blas VH (2015) Acute toxicity and risk assessment of three commercial detergents using the polychaete *Capitella* sp. C from Chetumal Bay, Quintana Roo, Mexico. Int Aquat Res 7:251–261

Editorial responsibility: Pablo Sánchez Jerez, Alicante, Spain

Submitted: March 14, 2018; Accepted: May 21, 2018
Proofs received from author(s): July 23, 2018